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Synthesis, characterization and chemisorption on gold of a β-cyclodextrin–lipoic acid conjugate

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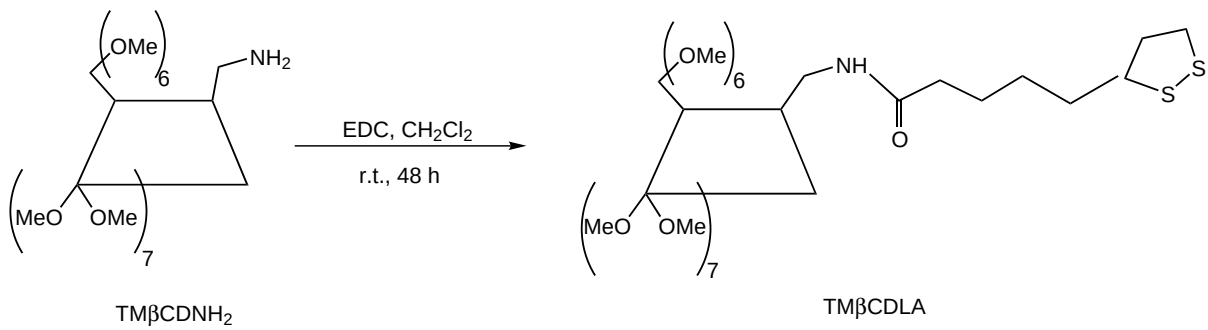
Abstract—Mono-6-lipoyl-amido-2,3,6-*O*-permethyl-β-cyclodextrin, TMβCDLA, was synthesized in 79% isolated yield by coupling the mono-6-amino-permethyl-β-cyclodextrin with lipoic acid in the presence of 1-(3-dimethylamino)ethyl carbodiimide. The identity of cyclodextrin–lipoic acid conjugate was confirmed by NMR spectroscopy and electrospray mass spectrometry. Chemiadsorption of TMβCDLA on colloidal gold was shown to occur by colloid flocculation test analysis.

Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides most commonly composed of six (α-), seven (β-), or eight (γ-) α-1,4-linked D-(+)-glucopyranose units arranged in a torous-shaped structure with a hydrophobic cavity that enables them to act as hosts for a variety of guest molecules.[1]

Combining the cyclodextrin binding ability with functional groups is of relevance in several areas to realize bio-mimetic models,[2] optical signal sensing of organic molecules,[3] vector-carrying molecules for controlled or site-specific delivery of drugs.[4] Deposition or attachment of properly functionalized CDs to solid surfaces is an attractive research area that may lead to quite interesting applications. In this field, recently much attention has been devoted to gold surfaces[5] and the present study is addressed to the synthesis of a CD derivative that, among other functions, can bind to gold. As a part of a project dealing with the preparation of cyclodextrin-functional group conjugates and, in view of the chemisorption properties of lipoic acid, here we report the synthesis and properties of a permethylated βCD bearing the above acid moiety linked via an amide bond: the mono-6-lipoyl-amido-2,3,6-*O*-permethylated-β-cyclodextrin, TMβCDLA.

As a rule for most applications, pure cyclodextrin derivatives rather than mixtures of positional isomers



TMβCDNH$_2$ TMβCDLA

Scheme 1. Synthesis of TMβCDLA.

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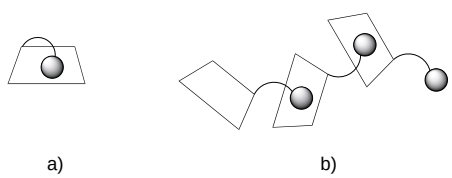
or homologous derivatives are needed. In the present case, we could exploit the availability of a starting molecule in which there is only one reactive functional group of the cyclodextrin while all the remaining hydroxy groups are blocked: the mono-6-amino permethylated β-cyclodextrin, TMβCDNH$_2$. Recently, Jicsinszky[6] designed and optimized a multigrams scale synthesis for TMβCDNH$_2$. Thus, the synthesis of TMβCDLA entailed the initial preparation of TMβCDNH$_2$ in four reaction steps starting from βCD: (1) mono-tosylation of the βCD/toluene complex in aqueous NaOH (34% yield); (2) formation of the 6-monoazido-6-monodeoxy-βCD by reaction of the tosylate with sodium azide in DMF (99% yield); (3) permethylation of the azide derivative with methyl iodide and sodium hydride in DMF (71% yield); (4) reduction of the azido group by catalytic transfer hydrogenation using hydrazine hydrate in the presence of Pd/C in methanol/water mixture (95% yield). The attachment of the lipoic acid to the cyclodextrin amino derivative via an amide bond was accomplished by using 1-(3-dimethyl amino)-ethyl-carbodiimide hydrochloride (EDC·HCl) as coupling reagent (Scheme 1).

TMβCDLA† was purified on silica gel using chloroform/isopropanol (95/5) as eluent (79% isolated yield). The ^{1}H NMR spectrum was recorded in $CDCl_3$ rather than D_2O in order to avoid intermolecular aggregation of TMβCDLA (vide infra). It showed the characteristic resonances of both cyclodextrin and lipoic acid moieties and the correct integration validated the 1:1 stoichiometry of the product. Furthermore, the presence of the amide proton resonance at 6.2 ppm and the absence of the amine proton signal at 1.5 ppm confirmed that TMβCDLA was indeed a cyclodextrin–lipoic acid conjugate and not merely an inclusion complex. Electro-Spray-ionization mass spectrometry (ESI-MS) analysis of TMβCDLA was carried out in methanol/water (1:1 v/v) in the presence of traces of ammonium chloride as cationizing agent. The base peak at $m/z=818.5$ corresponds to the bis-ammonium adduct of TMβCDLA. Other minor peaks were at $m/z=810$ ([TMβCDLA-$(NH_4)(H)]^{2+}$), 827.5 ([TMβCDLA$(NH_4)(H_2O)]^{2+}$), and 845.5 ([TMβCDLA $(NH_4)_2(H_2O)_3]^{2+}$).

Lipoic acid is well suited as a guest of cyclodextrins in water to give inclusion complexes with large binding constants.[7] Therefore, we expected that TMβCDLA could form both intra- and inter-molecular inclusion compounds as depicted in Scheme 2. A confirmation for the onset of aggregation phenomena came from the UV–vis spectrophotometric analysis. The Lambert–Beer plot for TMβCDLA in phosphate buffer (1 mM, pH = 7.0) in the presence of KCl (100 mM) was obtained by recording the absorbance at 333 nm versus the concentration of the cyclodextrin–lipoic acid conjugate. It showed that the Beer law was obeyed up to 5×10^{-5} M whereas, for more concentrated solutions, departure from the linearity becomes evident. This finding can be reasonably ascribed to the formation of *intermolecular* inclusion complexes involving TMβCDLA (Scheme 2b).



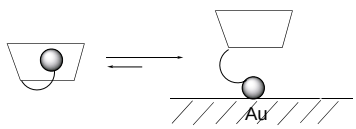
Scheme 2. Intra- (a) and intermolecular (b) inclusion complexes of TMβCDLA in aqueous solution.

† Selected analytical data for TMβCDLA: ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.6 (m, 6H, CH_2C), 1.8–2.0 (m, 3H, CH_2CHS), 2.3–2.6 (m, 2H, CH_2CO), 2.5 (t, 2H, CH_2S), 2.1 (s, 1H, NH), 3.0–4.0 (m, 102H, CH_2+OCH_3 of βCD), 5.0–5.4 (m, 7H, C1-protons βCD). TLC: $R_f=0.53$ (silica gel, $CHCl_3$/^{i}PrOH = 95/5 v/v).

Concerning the occurrence of an *intramolecular* inclusion of lipoic acid pendant group, the NMR analysis was incompatible with the low concentration of TMβCDLA needed to have exclusively *intramolecular* inclusion (concentration $<10^{-5}$ M). Thus we resort to an indirect kind of evidence by employing the *meso*-tetrakis (4-sulphonatophenyl) porphyrin, TPPS$_4$ as an external strong guest that may displace the built-in lipoic acid subunit. Thus, using conditions under which TPPS$_4$ effectively binds to the permethylated β-cyclodextrin[8] with revealing, conspicuous changes in the UV–vis spectra, the spectroscopic titration of TPPS$_4$ with TMβCDLA failed to detect any interaction. Since the water-soluble porphyrins employed are among the best guest for permethylated β-cyclodextrin, this suggests that the cyclodextrin cavity of TMβCDLA is not available for binding due to the strong self-inclusion of the lipoic acid moiety and such result apparently compromises the utility of TMβCDLA as a receptor.

However, it seemed to us that the binding ability of TMβCDLA could be restored by exploiting the strong chemiadsorption of lipoic acid on gold to enforce the opening of the self-inclusion complex (Scheme 3).

This idea us led us to investigate the chemiadsorption properties of TMβCDLA on colloidal gold surfaces. A solution of colloidal gold with 12–13 nm diameter particles was prepared according to the Natan's protocol[9] by the reduction of tetrachloroaurate ($HAuCl_4$) with trisodium citrate ($Na_3C_6H_5O_7$) as reducing agent in boiling water. The UV–vis spectrum of this solution displays the typical surface plasmon resonance absorption peak around 520 nm. Although colloidal solutions are relatively stable with respect to coagulation because the particles are negatively charged (due to absorption of anions) and they repel each other, the addition of electrolytes shields the charges on the gold



Scheme 3. Chemisorption of TMβCDLA on a gold surface.

particles and causes aggregation and/or precipitation of gold. Aggregation can be monitored by UV–vis spectroscopy by the concomitant red-shifting and decreasing of the plasmon resonance absorption signal.

A convenient way to stabilize colloidal solutions is by addition of high molecular adsorbates, like proteins, which inhibit aggregation due to the steric repulsion of particles and the screening of the particles from the ionic interactions that promote flocculation.[9] We expected that also TMβCDLA would be able to stabilize colloidal gold by coating the gold surface.

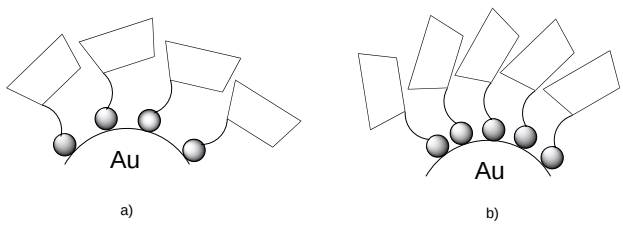
In order to establish the minimum amount of TMβCDLA needed to inhibit aggregation of colloidal gold, it was carried out a flocculation assay according the following protocol: Five aliquots of 4.25 nM solution of 12 nm diameter gold particles were incubated in disposable plastic cuvettes with increasing amounts of TMβCDLA and the resulting solutions allowed to stand in the dark for 3 h. Aqueous NaCl solution (100 μL, 1.0 M) was then added to each cuvette to induce aggregation and, 5 min later, the UV–vis spectra were registered (Fig. 1A). The lowest concentration of TMβCDLA examined (75 nM, colloidal gold: TMβCDLA = 1: 17.6) was insufficient to prevent gold particles aggregation. In fact, upon addition of NaCl, the color of the solution turned blue and after a few minutes on standing a dark precipitate of gold appeared. The UV–vis spectrum, registered before precipitation, showed a maximum at 640 nm (Fig. 1A, trace a) due to the collective plasmon resonance of the colloid aggregates.

Upon increasing the TMβCDLA concentration the stability of the colloidal gold solution increased as witnessed by the persistence of the plasmon resonance absorption at 520 nm after addition of the NaCl solution. A plot of the absorbance at 520 nm against the concentration of added TMβCDLA showed saturation behavior (Fig. 1B) and allowed to evaluate the minimum concentration of TMβCDLA needed to completely inhibit flocculation. This value was about 2 μM, which corresponds to about 470 molecules of TMβCDLA for gold particles.

Simple geometrical calculations based on a surface area of 452 nm^2 for the colloidal gold particles (spherical with 12 nm diameter) and on the size of the cyclodextrin ring (about 1 nm large and 0.6 nm deep), allow to estimate that up to 576 and 753 molecules of TMBCDLA can be accommodated on each gold particle assuming the two extreme situations shown in Scheme 4, the horizontal (a) and the vertical one (b), respectively.

Clearly, the experimental value of 470 molecules of TMBCDLA for gold particles is compatible with the above data but do not allow to speculate about the kind of arrangement of the cyclodextrin–lipoic acid conjugate on the gold surface. This topic is now being pursued by means of further reliable experimental evidence and refined modeling and calculations.

In conclusion, we have here reported the high yield synthesis of a covalent adduct between lipoic acid and $TM\beta CDNH_2$ via an amide bond. This product has been fully characterized by several experimental techniques. In aqueous solution TMβCDLA gives both inter- and intra-molecular aggregation as a result of the inclusion of the lipoic acid moiety the cyclodextrin cavity. TMβCDLA interacts with gold surfaces thanks to the strong chemisorption of lipoic acid allowing the



Scheme 4. Chemisorption of TMβCDLA on a colloidal gold surface: (a) horizontal disposition; (b) vertical arrangement.

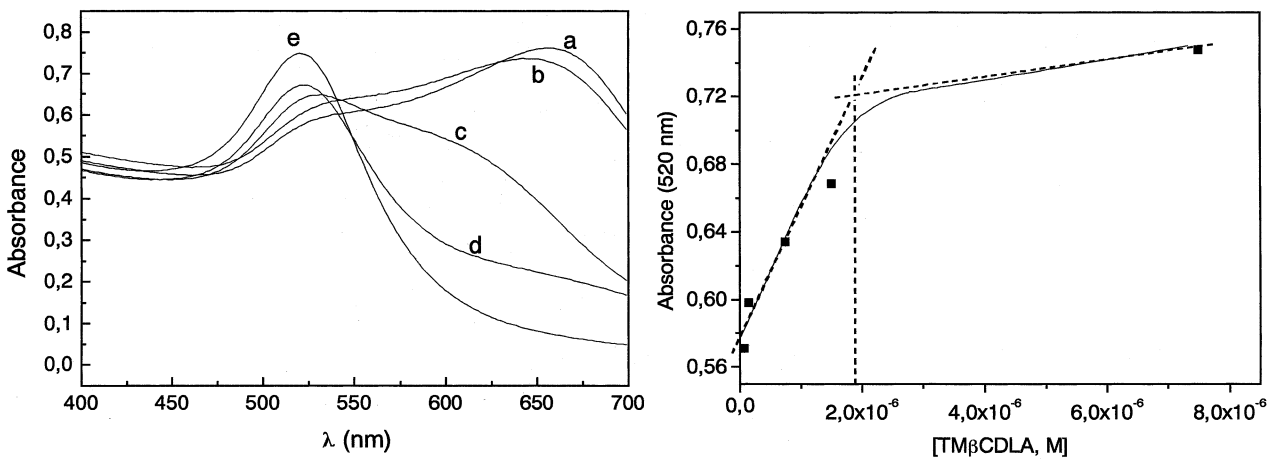


Figure 1. (A) UV–vis spectra for colloidal gold solutions 5 min after addition of NaCl (1.0 M, 100 μL). Spectra are shown for solutions containing: (a) 7.5×10^{-8} M; (b) 1.5×10^{-7} M; (c) 7.5×10^{-7} M; (d) 1.5×10^{-6} M; (e) 7.5×10^{-6} M of TMβCDLA. (B) Absorbance at 520 nm plotted against the concentration of TMβCDLA.

realization of nanosized particles coated with hundreds of permethylated CD molecules with a recovered binding ability of the cyclodextrin cavities.

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